II. REMARKS

Upon entry of the present amendment, claims 1 to 40 and 56 to 64 will be pending.

A. Regarding the Amendments

The "Brief Description" of each of Figures 3, 7, 10, 13B and 14 has been amended to include Sequence Identifiers for the sequences comprising a portion of adapter molecules and vector as shown in the respective Figures, and to correct typographical errors. The "Brief Description of Figure 4 has been amended to insert the Sequence Identifier for the pCR®2.1 vector, and to clarify that the nucleotide positions referred to in the "Brief Description" of Figure 4 are with respect to SEQ ID NO:17. Paragraphs 40, 51, and 52 also have been amended to refer to "the pCR® 2.1 vector", thus preserve the proprietary interest in the trademarked name of the vector. As such, the amendments merely address formalities or are supported by the specification, including the Figures, as originally filed, and do not add new matter.

Claims 41 to 55 are cancelled herein without disclaimer, and without prejudice to Applicants' pursuing prosecution of subject matter encompassed within one or more of the claims in an application claiming the benefit of priority of the subject application.

Claim 21 has been amended to correct a typographical error, wherein a single topoisomerase "site" was referred to in element c) of the claim instead of the "sites" at both ends as recited in element a). As such, the amendment does not add new matter.

B. Regarding the Drawings

The drawings are objected to because Figures 4, 5, 7, 10 and 12 to 14 contain sequences, but lack the required Sequence Identifiers. It is requested in the Office Action that proposed drawing corrections or corrected drawing be submitted.

Applicants point out that the Sequence Identifier for a sequence set forth in a drawing can be presented in either the drawing or in the Brief Description of the Drawings (MPEP 2422.02). In the present case, the Sequence Identifiers are set forth in the Brief Description of the

Drawings. In this respect, it is submitted that the Sequence Identifiers set forth in the Brief Description of Figures 5 and 12 are correct in the application as filed. In addition, the Brief Description of Figure 4 has been amended to insert the proper Sequence Identifier, and to clarify the reference to the recited nucleotide positions.

With respect to Figures 7, 10, 13B and 14, it is noted that the sequences shown in the Figures differ from the sequences of the adapters referred to by Sequence Identifiers in the "Brief Description of the Drawings" as filed due, for example, to the inclusion of restriction endonuclease recognition site sequences on the exemplified oligonucleotides. As such, Applicants have submitted herewith a Substitute Sequence Listing, which includes the additional sequences shown in Figures 7, 10, 13B and 14 as originally filed, and the Brief Descriptions for Figures 7, 10, 13B and 14 have been amended to include the Sequence Identifiers for these sequences.

In view of the amendments, it is submitted that the requirements for sequences set forth in Drawings have been met, and that new or corrected Drawings are not necessary. Accordingly, it is respectfully requested that this objection to the Drawings be withdrawn.

C. Regarding the Sequence Rules

The specification is objected to as not complying with the Sequence Rules, in that Figures 4, 5, 7, 10 and 12 to 14 contain sequences, but the Figures do not contain Sequence Identifiers. It is also noted in the Office Action that the CRF filed with the subject application could not be read by STIC.

A Substitute Sequence Listing, including paper copy and CRF, and the requisite Statements indicating that the Substitute Sequence Listing does not introduce new matter, have been submitted herewith, and the specification has been amended as discussed in Section B, above. Accordingly, it is respectfully requested that this objection to the specification be withdrawn.

D. Rejections under 35 U.S. C. § 112

The rejections of claims 52 and 54 under 35 U.S.C. § 112, second paragraph, as allegedly vague and indefinite, are respectfully traversed.

Although Applicants traverse this rejection of the claims, it is noted that claims 52 and 54 have been cancelled without prejudice. As such, it is submitted that this issue is moot and, therefore, is respectfully requested that the rejections of claims 52 and 54 under 35 U.S.C. § 112, second paragraph, be removed.

C. Prior Art Rejections

The rejection of claims 1 to 51 and 53 to 64 under 35 U.S.C. § 102(e) as allegedly anticipated by Heyman et al. (U.S. Publ. No. 2001/0044137) is respectfully traversed. It is noted that claims 41 to 55 have been cancelled. As such, the rejection is addressed with respect to claims 1 to 40 and 56 to 64.

The present invention is directed to methods and compositions useful for linking nucleic acid molecules via strand invasion (see, e.g., paragraph 84, pages 33-34). For purposes of illustration, the Examiner's attention is directed to Figure 1A, which exemplifies compositions useful for practicing a strand invasion method (see, also, paragraphs 32 and 33, pages 14-15). Figure 1A shows a first double stranded (ds) nucleic acid molecule having a 5' overhang, and a second ds nucleic acid molecule having a blunt end; the "boxes" indicate the 5' portion of the second ds nucleic acid molecule and the complementary 5' overhang of the first ds nucleic acid molecule, which are at the ends to be joined. Strand invasion proceeds by the 5' overhanging portion of the first ds nucleic acid molecule displacing the 3' terminal sequence of the second ds nucleic acid molecule, thus positioning, with reference to the boxed regions in Figure 1A, the 3' terminus of the first ds nucleic acid molecule adjacent to the 5' terminus of the second ds nucleic acid molecule. Where, according to a method of the invention, a topoisomerase is covalently bound at the 3' terminus of the first ds nucleic acid molecule, which is adjacent to the

5' terminus of the second strand, the topoisomerase can link the 3' terminus of the first strand to the 5' terminus of the second strand, thus generating a directionally linked ds recombinant nucleic acid molecule (see, e.g., paragraphs 85-87, pages 34-35).

In brief, claims 1, 21, 34, 56 and 62 are directed to methods of using strand invasion to covalently link double stranded (ds) nucleic acid molecules, and to compositions and kits useful for practicing such methods. More specifically, the claimed methods require, in part, a first ds nucleic acid having at a first end a 5' overhang (claims 1 and 34) or a 5' target sequence (claim 21) and a second ds nucleic acid having a first blunt end, wherein the blunt end comprises a 5' nucleotide sequence complementary to the 5' overhang (claims 1 and 34) or 5' target sequence (claim 21). The claimed compositions require, in part, a combination of ds nucleic acid molecules that includes 1) a first ds nucleic acid having at a first end a 5' overhang and 2) a second ds nucleic acid having a first blunt end, wherein the blunt end comprises a 5' nucleotide sequence complementary to the 5' overhang (claim 56). The claimed kits require, in part, 1) a ds nucleic acid molecule having a first topoisomerase covalently bound at the 3' terminus of a first end and a second topoisomerase covalently bound at the 3' terminus of the second end, wherein the first end further comprises a 5' overhang, and 2) a plurality of second ds nucleic acid molecules, each comprising a first blunt end comprising a 5' nucleotide sequence complementary to the first 5' overhang of the first ds nucleic acid molecule (claim 62).

It is stated in the Office Action that Heyman et al. describe a kit, composition, and method for generating a directionally linked recombinant nucleic acid molecule, thus anticipating the claimed subject matter (citing generally to paragraphs 13-21, 28-35, 43-51, 55-60, 66-84, and 173-178; Office Action, page 5). However, Heyman et al. do not teach or suggest compositions or methods for directionally linking nucleic acid molecules by strand invasion and, more specifically, the reference does not teach or suggest compositions containing, or methods using, a first ds nucleic acid having at a first end a 5' overhang (or a 5' target sequence) and a second ds nucleic acid having a first blunt end, wherein the blunt end comprises a 5' nucleotide sequence

complementary to the 5' overhang (or 5' target sequence). As such, Heyman et al. do not anticipate the subject matter of claims 1 to 40 or 56 to 62 and, therefore, it is respectfully requested that the rejection of the claims under 35 U.S.C. § 102(e) as allegedly anticipated by Heyman et al. be removed.

The rejection of claims 1 to 20 under 35 U.S.C. § 102(e) as allegedly anticipated by Hodgson (U.S. Publ. No. 2002/0025561) is respectfully traversed.

It is stated in the Office Action that Hodgson describes a method for generating a directionally linked recombinant nucleic acid molecule, thus anticipating the claimed subject matter (citing generally to paragraphs 14-23, and 38-48; Office Action, page 5). Applicants point out that Hodgson mentions "topoisomerase I" one time in the cited application (paragraph 38), where it is noted that T/A cloning can be used to insert a PCR amplification product having a single 3' A overhang into a vector provided with topoisomerase. As shown in the attached Exhibit A, a topoisomerase charged vector useful for T/A cloning contains the topoisomerase covalently linked to a 3' T overhang, wherein the T overhang on the vector and an 3' A overhang on the PCR product facilitates joining of the vector and PCR product (Exhibit A, pages 3 and 4 of 2001 Invitrogen Corp. catalog; see Figure 1 on each of pages 3 and 4).

It is submitted, however, that Hodgson does not teach or suggest strand invasion methods using a first ds nucleic acid having at a first end a 5' overhang (or a 5' target sequence) and a second ds nucleic acid having a first blunt end, wherein the blunt end comprises a 5' nucleotide sequence complementary to the 5' overhang (or 5' target sequence). As such, Hodgson does not anticipate the subject matter of claims 1 to 20 and, therefore, it is respectfully requested that this rejection of claims 1 to 20 under 35 U.S.C. § 102(e) be removed.

The rejection of claims 1 to 51 and 53 to 61 under 35 U.S.C. § 102(e) as allegedly anticipated by Shuman (U.S. Pat. No. 6,548,277) is respectfully traversed. It is noted that claims 41 to 55 have been cancelled. As such, the rejection is addressed with respect to claims 1 to 40 and 56 to 61.

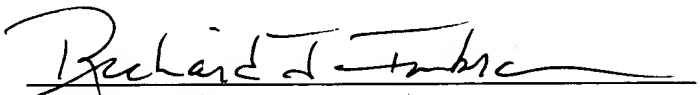
It is stated in the Office Action that Shuman describes a composition and method for generating a directionally linked recombinant nucleic acid molecule, thus anticipating the claimed subject matter. As with the other cited references, however, Shuman does not teach or suggest the subject matter of the claims under examination. As such, it is submitted that the reference does not anticipate the subject matter of claims 1 to 40 or 56 to 61 and, therefore, is respectfully requested that the rejection of the claims under 35 U.S.C. § 102(e) as allegedly anticipated by Shuman by removed.

It is submitted that the claims are in condition for allowance, and a notice to that effect is respectfully requested. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to the subject application.

Please charge any additional fees, or made any credits, to Deposit Account No. 50-1355.

Respectfully submitted,

Date: October 23, 2003



Richard J. Imbra
Registration No. 37,643
Telephone: (858) 677-1496
Facsimile: (858) 677-1465

USPTO CUSTOMER NUMBER 28213
GRAY CARY WARE & FREIDENRICH LLP
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133

Enclosure: Exhibit A

Description of TOPO® Cloning

Topoisomeras Greatly Improv s Ligation

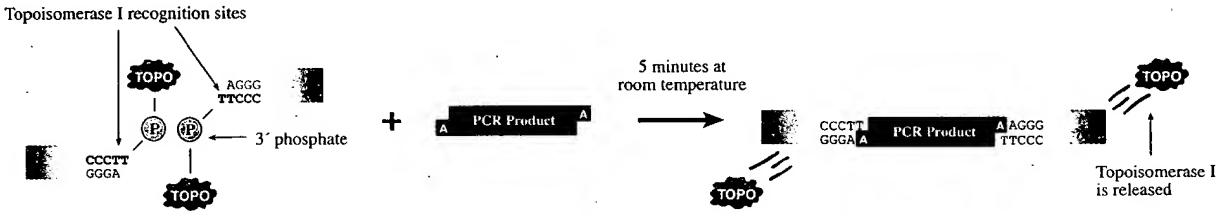
TOPO® Cloning makes cloning PCR products and other DNA molecules faster and more successful. It enables five-minute, bench-top ligation and yields ≥95% recombinants. You'll not only get your clones faster than with ligase-mediated methods, you won't waste additional time repeating experiments that didn't work the first time. The key to TOPO® Cloning is the enzyme DNA topoisomerase I, which functions like both a restriction enzyme and a ligase. The typical role of this enzyme is to cleave and then rejoin DNA during replication. *Vaccinia* virus topoisomerase I recognizes the specific pentameric sequence 5′-(C/T)CCTT-3′ and forms a covalent bond at the phosphate group of the 3′ thymidine. It then cleaves one strand of the DNA, enabling the DNA to unwind around the axis of the uncleaved strand. The enzyme then religates the ends of the cleaved strand and is released from the DNA.

Topoisomerase is Better than Ligase

TOPO® Cloning takes advantage of the religating activity of topoisomerase I to enable five-minute ligation of DNA molecules that do not contain a 5′ phosphate. TOPO® vectors are provided linearized with topoisomerase I covalently bound to the 3′ phosphate on each strand, so they readily ligate PCR products with compatible ends (Figure 1). TOPO® Cloning provides several advantages over ligase-mediated methods:

- Ligase is often contaminated by nucleases that can nick the DNA and vector ends, reducing the number of recombinants
- Topoisomerase I protects the vector ends from exonucleases to maintain the integrity of the vector
- Only two molecules must come into contact for ligation to occur–the TOPO®-activated vector and the insert. This is a much higher probability event than a typical ligation in which three molecules–the vector, the insert, and ligase–must interact for ligation to occur

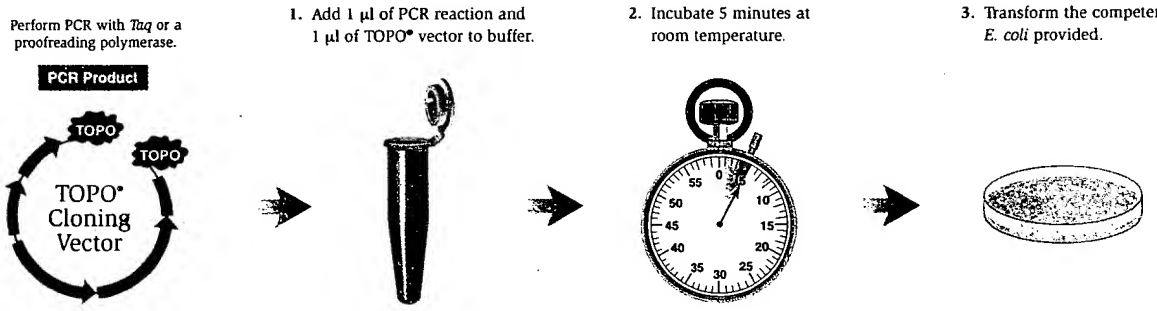
Figure 1 - The TOPO® Cloning mechanism



Thr e Simple Steps

TOPO® Cloning requires just three simple steps. All you need to do is combine your PCR reaction and TOPO® Cloning vector in a salt buffer, wait five minutes, and then transform (Figure 2).

Figure 2 - The TOPO® Cloning protocol



Important Licensing Information

TOPO® Cloning is covered under U.S. Patent No. 5,766,891, other patents pending, and corresponding foreign patents pending and is for the purchaser's internal research only. Such use is limited to the cloning of nucleic acids as described in TOPO® Cloning manuals and specifically excludes, without limitation, resale or the making or selling of any commercial product or service. All non-research uses or applications of TOPO® technology require a license from Invitrogen.

Exhibit A

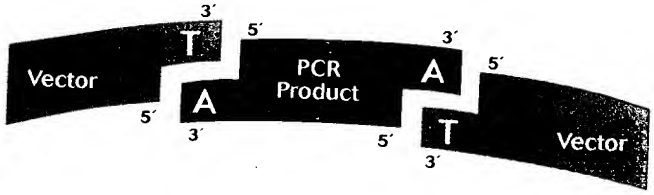
chapter 1 cloning

Description of TA Cloning®

TA Cloning® Simplifies PCR Cloning

The TA Cloning® technology was designed to clone PCR products produced by *Taq* polymerase (and certain polymerase mixtures). It takes advantage of the terminal transferase activity of these polymerases, which adds a single, 3´-A overhang to each end of the PCR product. A of Invitrogen's TA Cloning® and TOPO TA Cloning® vectors are supplied linearized with single, 3´-T overhangs. This enables the direct liga tion of PCR products at high efficiencies (Figure 1).

Figure 1 - The TA Cloning® concept



TA Cloning® significantly improves the cloning of PCR products because it:

- Eliminates the need to synthesize PCR primers that contain restriction sites
- Does not require any post-PCR modification, like digestion, blunting, or dephosphorylation
- Eliminates gel or column purification of PCR products prior to ligation
- Does not require ligation of adapters to the PCR products

The TA Cloning® and TOPO® Cloning technologies have been combined to create the TOPO TA Cloning® Kits. TOPO®-activation improves both the speed and effectiveness of the TA Cloning® method (Table 1).

Table 1 - Comparison of TA Cloning® and TOPO TA Cloning®

	TA Cloning® Kits (page 13)	TOPO TA Cloning® Kits (page 8)
Results	≥80% recombinants	≥95% recombinants
Ligation time	4 hours (preferably overnight)	5 minutes

invitrogen

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catalo